MORPHOLOGY OF THE GENUS ACTINOMYCES. II

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(WITH PLATES II–IX)

Taxonomy of species

The obscurity that has surrounded the morphology of *Actinomyces*, besides involving the genus in improbable speculations concerning its phylogenetic relationship to the bacteria, has brought about also a most unfortunate condition in the taxonomy of the many described species. Most of the work on the genus has been done by investigators with bacteriological inclinations, and even where this has not been true, the prevalent view of the nature of these minute plants has lead to the adoption of methods scarcely applicable to mycological research. A discussion of characteristics like the occurrence of endospores, flagella, capsules, sheaths, and involution forms, cannot be regarded as constituting a morphological treatment more satisfactory for species of *Actinomyces* than for species of *Mucor* or *Boletus*.

The dependence of certain biochemical processes, particularly chromogenesis, on definite conditions of nutrition, and the conspicuous differences resulting from comparatively slight changes in the substratum, have long been noted by students of *Actinomyces*, yet descriptions of new species have continued to appear, based largely and often quite exclusively, on these variable activities. Very frequently writers have not compared their organisms with others reported by previous investigators; and in recent years there has been a tendency to disregard altogether the taxonomic contributions of the preceding decades. Moreover, while identities have frequently not been recognized where they existed, in other cases organisms have been supposed to be identical on extremely slight evidence. One of these cases that has led to an unusual measure of confusion is that of GASPERINI'S *Actinomyces chromogenus*. This species was identified by both GASPERINI and ROSSI-DORIA (19) with an organism isolated from the air by the

latter and designated as *Streptothrix nigra*. In culture it was characterized by a dark brown or black discoloration of certain kinds of substrata, a reaction easily obtained on potato agar, for example, and ascribed by LEHMAN and SANO (13) to the production of tyrosinase. Until recently it has been the custom among writers to refer nearly every member of the genus showing a tyrosinase reaction to *Actinomyces chromogenus*, LUTMAN and CUNNINGHAM (14) going so far as to identify this species with the potato scab organism. This practice, which would unite forms so different in appearance and method of development as, for example, *Actinomyces* I and III, regardless of pronounced differences in size and in dextrorse or sinistrorse condition, is not defensible on morphological grounds. KRAINSKY resolved the "chromogenus" complex into 4 species; while WAKSMAN and CURTIS increased the number of derivatives to 8. Of the 17 morphologically distinct saprophytic species figured in this paper, 11 exhibit a tyrosinase reaction; and these represent less than one-fifth of the number of similarly active species which the writer had occasion to examine.

The genus awaits the attention of an investigator in a position to make a comprehensive study involving at least the larger proportion of species existing within wide geographical ranges. The summaries given later, of the more important facts about each species selected for morphological treatment, are not to be regarded as descriptions intended for taxonomic purposes.

ACTINOMYCES I

CULTURAL CHARACTERS.—On glucose agar (0.5 g. peptone, 10.0 g. glucose, 20.0 g. agar, 1000 cc. tap water) nutritive mycelium of individuals smooth, opalescent, more or less confluent; sporulation moderately slow and commencing as a light creamy zone near the periphery; no diffusible stain. On potato agar (decoction of 200 g. peeled potatoes, 2.0 g. glucose, 20.0 g. agar, 1000 cc. water) nutritive mycelium light olivaceous; sporulation moderately abundant, the raised areas where the yellowish gray fructifications are to appear being previously distinguishable by a deep brownish green coloration; guttation never copious, often absent; tyrosinase reaction moderate, but distinct.

Morphology.—The development of the erect sporogenous hyphae of this species is strictly successive, and may be followed in the branch *d* in fig. 2, the younger of any 2 hyphae being distinguishable by its attenuated attachment. The partly disrupted chain of spores *d1* here represents the original prolongation of branch *d;* the chain *d2* represents a secondary branch, the spores here being mature but still retaining their spiral disposition without showing indications of disruption; while *d3* represents a tertiary branch, in which septation has not commenced. A similar sequence is illustrated in the succession of derivatives *b1*, *b2*, *b3*, and *b4* from the branch *b*, as well as in the 5 elements *c1–c5* associated with the branch *c*.

A more complex system of fertile hyphae is shown in fig. 1, but the larger fructifications are probably 10 or even 15 times more extensive, and bear many thousands of spores. The species is characterized by close sinistrorse spirals, of 2–6 turns, and 3–4 μ in diameter, which during the later stages of maturation are relaxed, although indications of them may persist in the flexuous or sinuous course of the mature chains of spores. The mature spores are ellipsoidal, 1–2-nucleated, with a distinctly visible wall and a central vacuole of varying size. They measure $1.2–1.4 \times 1.4–20$ μ, and upon germinating produce 2–4 germ tubes, which early proliferate numerous branches, and show at intervals some dark staining granules.

Isolated 5 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES II

Cultural characters.—On glucose agar, growth moderate; nutritive mycelium colorless, early covered with a cretaceous or downy aërial mycelium; pigment absent. On potato agar, development of nutritive mycelium moderately rapid; aërial mycelium appearing in scattered areas, first white, later becoming slightly discolored; substratum stained yellow by a soluble pigment; tyrosinase reaction absent.

Morphology.—The most conspicuous feature of this species is the extraordinary thickness of the septa ($0.3–0.35$ μ) associated

with spore production, and their insertion at distensions in the sporogenous hyphae. A comparison of branch *c1* with the younger branch *c2* (fig. 5) corresponding to the conditions shown diagrammatically in figs. 8*b* and 8*c* respectively, shows that the growth in thickness of the hyphae takes place subsequent to the appearance of the septa. After the filament has attained its growth, the septa split along a median plane (figs. 5*a*, 5*c1*, 8*d*), and the 2 halves are drawn apart by the contraction of the delimited protoplasts. Further maturation occurs in the distribution of the deeply staining wall substance, in the strengthening of the peripheral wall, and by an enlargement of the latter, the elimination of the median, hourglass-like constriction of the spore, resulting finally in an approximately cylindrical structure measuring $0.7–0.9 \times 0.8–1.1\ \mu$.

The terminal and the basal spores of each chain retain a somewhat asymmetrical shape, owing to the absence of the massive septum at one of their ends. By an apparently abnormal development, metachromatic granules may be formed in the spores derived from some hyphae, resulting in a condition illustrated in the lowest spore in fig. 8*f*.

The axial filaments are represented by long prostrate hyphae, branching at irregular, long intervals. Septation is confined to the fertile branches. The sterile hyphal portions below the sporogenous terminations taper gradually toward their attenuated attachments. Development and sporogenesis near the axial terminations are successive, and involve the formation of close sinistrorse spirals of 1–5 turns, $3.5–6.0\ \mu$ in diameter.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES III

A. lavendulae Waksman and Curtis

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium slightly yellow on reverse side, central area completely covered with velvety aërial mycelium, first white but gradually assuming a beautiful lavender shade; no soluble stain. On potato agar, growth very profuse; mycelium abundant, changing from white to lavender; guttation moderate to profuse; tyrosinase reaction vigorous.

Identity with *A. lavendulae* established by comparison of cultural and morphological characteristics.

Morphology.—The mycelium consists of long prostrate axial filaments, branching rarely except at the end. Sporulation is usually initiated at the tip of the filament, and proceeds basipetally by the insertion and transformation of almost invisible septa, to the point of attachment of the first sporogenous branch (figs. 10, 11). The sporogenous branches are rarely crowded, although at the base of the sporogenous axial termination an opposite arrangement is not uncommon. Secondary branching occurs frequently; septa are entirely absent, except when associated with the progressive basipetal delimitation of spores.

The sporogenous hyphae terminate in dextrorse, moderately compact spirals of 4–12 turns, 2.0–3.8 μ in diameter. The spores are ellipsoidal, with nuclei not readily demonstrable. Metachromatic material occurs abundantly in many old hyphae (fig. 15).

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES IV

Cultural characters.—On glucose agar, nutritive mycelium colorless; aërial mycelium moderately profuse, velvety in appearance, changing from white to smoky blue; no guttation. On potato agar, tyrosinase reaction vigorous; aërial mycelium first produced white, not subsequently much discolored, becoming matted to the substratum as a result of excessive guttation, and later completely overgrown by a loose growth of smoky blue secondary mycelium.

Morphology.—The sporogenous branches with dextrorse spirals of 2–12 open turns, 1.5–2.5 μ in diameter, are attached to the long axial filaments usually at wide intervals, in a loose racemose arrangement. Secondary branching, although rare, occurs occasionally, and is then associated with simultaneous sporulation (fig. 18). Development of the 1–2-nucleated spores, 0.7–0.8×0.9–1.1 μ, proceeds by the insertion of conspicuous septa, followed by their constriction and subsequent conversion to hyaline isthmuses (figs. 70*a–e*). Two germ tubes are usually produced, of a more or

less uniform diameter, and proliferating branches at relatively wide intervals.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES V

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium on reverse side slightly yellowish; the surface completely covered with a luxuriant velvety or cottony weft of pinkish-yellow aërial mycelium; guttation slight. On potato agar, nutritive mycelium chocolate-colored, firm lichenoid, crimped around margin; aërial mycelium, as on glucose agar, but less profuse; tyrosinase reaction vigorous.

MORPHOLOGY.—The fertile hyphae, which are attached to prostrate axial filaments at long intervals, are terminated by relatively close sinistrorse spirals of 4–12 turns 2.0–4.0 μ in diameter, developing spores (0.6–0.8×0.9–1.1 μ), like *Actinomyces* IV, by the insertion of conspicuous septa, followed by their constriction and conversion. A peculiar characteristic is found in the sterilization of the basal portion of the fertile hyphae, by an apparent abortion of its lower potential spores.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VI

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, completely covered with a felty aërial mycelium, first white, later assuming a deep smoky tinge. On potato agar, nutritive mycelium excessively wrinkled, partially covered with a slightly discolored aërial mycelium; tyrosinase reaction vigorous.

MORPHOLOGY.—The species appears closely allied to *Actinomyces* V, differing from the latter chiefly in the absence of any evidence of sterilization, and in the shorter length of its sinistrorse sporogenous spirals, which consist of only 2–6 turns, 2.0–4.0 μ in diameter. The spores are uninucleated, measure 0.7–0.8×0.9–1.1 μ, and are developed by the insertion and transformation of conspicuous septa. Fertile hyphae are attached to the axial fila-

ments with considerably greater frequency, and secondary branching, characterized by the successive type of development, is common.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, early developing an aërial mycelium from the center outward, the latter changing from white to light gray with increasing age. On potato agar, nutritive mycelium luxuriant, developing rapidly; aërial mycelium represented by a slight cretaceous development toward the top of the slant; tyrosinase reaction vigorous.

MORPHOLOGY.—This species departs from the main trend of the 3 preceding forms in the relatively close arrangement of its branches on the axial filament, and in the elaboration of these branches by further ramifications in a typically successive order. Nearly spherical to ellipsoidal spores, $0.6–0.8 \times 0.7–1.0\ \mu$, are produced from moderately close sinistrorse spirals of 3–8 turns $2.0–3.0\ \mu$ in diameter, by the development represented in fig. 70*a–e*, but the septa are relatively thin, and occasionally fall below the limit of clear visibility.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VIII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium nearly colorless, secreting a diffusible yellow pigment; aërial mycelium moderately profuse, velvety, first white, later changing to a light bluish color. On potato agar, growth similar, but soluble pigment absent; no tyrosinase reaction.

MORPHOLOGY.—The fertile hyphae of this species may be attached to the axial filaments in a diffuse racemose arrangement (fig. 46), or crowded in a compact capitate system. The swellings in the axial filaments in figs. 43, 44, 45, and 46 at the bases of sporogenous branches indicate the mode of origin of the *Leptomitus*-like distensions shown in figs. 47 and 48.

The small, ellipsoidal, uninucleated spores, 0.5–0.6×0.6–0.8 μ, are formed from close, sinistrorse spirals of 2–10 turns 1.2–2.5 μ in diameter. Indications of septa can be seen only rarely. The mature spore chains upon collapsing cohere in irregular zooglœa-like masses, a peculiarity of behavior dependent probably on a gelatinization of wall material. Upon germination, 1 or 2 tubes are produced, relatively thick and abundantly branching.

Isolated 6 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES IX

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, forming no soluble pigment; aërial mycelium at first white, becoming light smoky blue in the course of a few days. On potato agar, cultural characters similar, but growth more profuse, guttation moderate, discoloration of aërial mycelium more rapid; tyrosinase reaction absent.

MORPHOLOGY.—The most characteristic feature of this species is the greater thickness of the fertile hyphae below the second turn of the spiral. The latter are sinistrorse, usually with very close turns, varying in number from 1 to 16, and measuring 1.5–2.0 μ in diameter. They give rise to ellipsoidal, uninucleated spores, 0.5–0.7×0.6–1.0 μ, without the appearance of clearly visible septa. It seems highly probable that cross-walls nevertheless occur, since occasionally a median partition may be differentiated in the hyaline attentuated connections between two spores (fig. 51), suggesting a development similar to that indicated in fig. 70*a–e*.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES X

Streptothrix alba Rossi-Doria; *Actinomyces griseus* Krainsky (?)

CULTURAL CHARACTERS.—On glucose agar, growth poor and not characteristic. On potato agar, growth excessively rapid, nutritive mycelium colorless; aërial mycelium firm, white, changing rapidly to a yellowish gray; secondary growth occurring in the formation of numerous successive rings of sporodochia, or in the development of cottony white masses of mycelium from below the thick

crust of old mature spores; tyrosinase reaction absent; substratum stained a faint greenish yellow in old cultures.

MORPHOLOGY.—According to WAKSMAN and CURTIS, the aërial filaments of this species possess only a slight tendency to branch. The writer was led to a somewhat different conclusion, as the axial hyphae are usually found to proliferate fertile branches at moderately close intervals. Occasionally, as in fig. 58, indications of a successive sequence may be observed, but more frequently the development of the different elements of a ramifying system occurs without any recognizable interrelation. The short, cylindrical spores, 0.7×0.7–$1.0\ \mu$, are formed, as in *Actinomyces* XVI, by a septation of the fertile hyphae, followed by splitting of the partitions along a median plane, but the septa are usually less conspicuous, and often not clearly visible, and the fertile hyphae show no indication of a spiral condition.

A striking dimorphism characterizes the mycelium of this species, as well as that of a number of other forms observed by the writer. The deeper sterile aërial hyphae below the sporogenous layer typically are extremely minute, with a diameter frequently not exceeding $0.3\ \mu$; their protoplasmic contents show little affinity for stains; and the contours of their walls are uniformly smooth. The more superficial hyphae, which usually attain a thickness of $1.0\ \mu$, and are distinguishable by markedly irregular contours, contain dense deep staining protoplasm; and when septa are present, they are sometimes associated, as in *Actinomyces* XVII and XVIII, with spherical structures. The thicker filaments bear the sporogenous branches, and, in general, appear to constitute the expanded prolongations of the minute hyphae (fig. 59).

Isolated twice from soil collected in Cambridge, Massachusetts; once from tap water; very frequently from outdoor air; several times from gross cultures of dead leaves; 4 times from horse dung undergoing fermentation at 50–60° C.

SYNONOMY.—In his description of *Streptothrix alba*, ROSSI-DORIA records two characteristics that establish its identity beyond much danger of confusion: a conspicuous preponderance in number over any of its congeners on plates exposed to the air, and a tendency toward the formation of concentric rings more pronounced than that of any other species. ROSSI-DORIA

attributed this preponderance in the air to its omnivorous character, enabling it to develop on a large variety of substrata, "Questa *Streptothrix* cresce, si può dire, dappertutto, tanto su terreni di natura vegetale quanto su terreni di natura animale. E per ciò nonchè la grande sua produzione di spore, che essa si trova cosi diffusa nell'aria ed altrove. Pare che essa possa svilupparsi anche nel terreno." In spite of this fortunate and quite distinctive characterization, the specific term "albus" subsequently came to be used in a manner as miscellaneous as "chromogenus," being applied generally to any type with a light mycelium showing no tyrosinase reaction.

The same species was treated in the publication of WAKSMAN and CURTIS as *Actinomyces griseus* Krainsky. I have not been able to satisfy myself fully about the identity of KRAINSKY'S organism; nor would it seem possible to reach any definite conclusion without an examination of authentic material.

ACTINOMYCES XI

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium first colorless, becoming slightly reddened with increasing age; aërial mycelium first white, rapidly changing to a bluish violet. On potato agar, nutritive mycelium gradually becomes deep red by the slow accumulation of a slightly diffusible pigment; tyrosinase reaction absent.

MORPHOLOGY.—More or less erect fructifications are developed along the distal portions of long prostrate filaments. Branching is abundant and only occasionally shows indications of a successive sequence. The aërial hyphae in the dendroidic structures (figs. 64, 66) are often conspicuously vacuolate, especially in the inflated distensions from which a number of fertile branches arise. The latter terminate in sinistrorse spirals of 4–6 turns, $2.0–3.0\ \mu$ in diameter, from which, by the insertion of conspicuous septa and their subsequent transformation to hyaline isthmuses, spores $0.5–0.7 \times 1.0–1.2\ \mu$ are produced.

Isolated once from soil collected in Cambridge, Massachusetts; identical with an organism isolated by Mr. H. J. CONN from soil collected near Geneva, New York.

ACTINOMYCES XII

A. aureus Waksman and Curtis

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium yellowish on reverse side; aërial mycelium changing from white to pale yellowish gray; soluble stain absent. On potato agar, nutritive mycelium darker on reverse side; aërial mycelium more profuse, forming a somewhat more deeply colored felty layer; tyrosinase reaction moderate. Identity with *Actinomyces aureus* established by comparison with authentic material of the latter.

MORPHOLOGY.—In this species long prostrate filaments terminate in more or less erect fructifications. Secondary branches are proliferated from the lateral elements, generally in successive sequence. A more or less pronounced cuneate thickening of the hyphae below the insertion of a branch is characteristic of the species. The ellipsoidal, uninucleated spores, $0.5–0.7 \times 0.8–1.2\ \mu$, are formed by the insertion of conspicuous septa in open, sinistrorse spirals of 2–7 turns, $3.0–4.0\ \mu$ in diameter.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XIII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium light orange-brown, the separate individuals fused into a massive pellicle with a depressed, crimped margin. On potato agar, nutritive mycelium dark chocolate-brown, wrinkled, lichenoid, secreting a diffusible red pigment; tyrosinase reaction absent. Aërial mycelium on both substrata loose, cottony; developing slowly, first white, later changing to a dull bluish tint.

MORPHOLOGY.—The aërial mycelium consists of extremely long filaments, which rarely show any evidence of branching (figs. 74 75), and toward their terminations follow an undulating or slightly spiral course. Sporulation occurs as the result of protoplasmic contractions without the appearance of visible septa, the chains of cylindrical spores, $0.4 \times 1.2–1.6\ \mu$, being held together for some time by the evacuated portions of hyphal wall, that seem to undergo no apparent constriction.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XIV

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium usually colorless, but frequently becoming deep brown or black; aërial mycelium consisting of a dense velvety weft, first white, later changing to a creamy yellow. On potato agar, growth similar; tyrosinase reaction absent.

MORPHOLOGY.—This species is characterized by the production of extensive prostrate fructifications through the proliferation of numerous lateral branching processes from long axial filaments (figs. 76, 79, 81). A septum is occasionally present immediately above the attachment of a branch, but more frequently is absent. Secondary ramifications, resulting in more or less complex elements, take place without reference to the stage of sporogenesis in the proliferating branch. The ellipsoidal uninucleated spores, 0.5–0.7×0.8–$1.2\ \mu$, are derived from sinistrorse spiral hyphae of 1–8 turns, 2.0–$4.0\ \mu$ in diameter, by the insertion and transformation of relatively thin septa, or without the appearance of demonstrable septa.

Isolated 4 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XV

CULTURAL CHARACTERS.—On glucose or potato agar, nutritive mycelium opalescent; aërial mycelium first white, becoming only slightly discolored with age; tyrosinase reaction moderate.

MORPHOLOGY.—Microscopically this species closely resembles *Actinomyces* IV, differing from the latter chiefly in the abundant proliferation of branches of the second or of a higher order. The lateral elements thus formed follow the successive type of development (figs. 82, 83). The uninucleated spores, 0.7×0.9–$1.0\ \mu$, are formed from dextrorse spiral hyphae of 3–12 turns, 1.8–$2.5\ \mu$ in diameter, by the constriction of conspicuous septa, and their transformation into hyaline isthmuses.

Isolated twice from soil collected in Manhattan, Kansas.

ACTINOMYCES XVI

CULTURAL CHARACTERS.—On glucose agar, growth very meager; never producing an aërial mycelium. On potato agar, develop-

ment rapid; nutritive mycelium dark brown or greenish brown; aërial mycelium profuse, changing from white to violet or pinkish gray; guttation profuse; tyrosinase reaction moderate.

MORPHOLOGY.—In this species the characteristic development consists in the proliferation of a number of long branches in an irregular whorl from a long and somewhat thickened axial filament. Secondary branching is common, but usually more or less remote. Vacuoles associated with hyphal distensions are found in the axial filaments and in the main branches, and metachromatic granules occur abundantly in many of the older sterile hyphae (fig. 91). The long cylindrical spores, $0.6–0.7 \times 1.0–2.0\ \mu$, are formed by the septation of sporogenous hyphae that terminate in open, sinistrorse spirals of 2–3 turns, $4.0–5.5\ \mu$ in diameter, followed by the splitting of the septa along a median plane, and the separation of the two halves by a contraction of the delimited protoplasts. The progress of sporogenesis is usually basipetal, but not infrequently the first divisions may result in a number of segments of varying lengths, which by subsequent divisions are reduced to the magnitude of the ultimate spores.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XVII

A. scabies (Thaxter) Güssow (6)

MORPHOLOGY.—The aërial mycelium of this species, which is one of the largest of dextrorse forms, consists of long prostrate filaments on which lateral branches are inserted at short intervals. Secondary branching is abundant and usually associated with a successive order of development (figs. 93*a1–a3*). The more or less cylindrical spores, $0.8–0.9 \times 1.3–1.5\ \mu$, are developed from dextrorse spiral hyphae of 3–14 turns, $2.0–3.5\ \mu$ in diameter, by the insertion of conspicuous septa and their subsequent splitting along a median plane. In many hyphae the septa before their division can be seen to occupy a transverse equatorial position in the peculiar spherical structures to which reference has been made elsewhere, and which here occupy slight but perceptible hyphal distensions (figs. 92, 93*h1*, 93*h2*, 101*cy*). Whenever the spherical structures

are absent, the fertile hyphae are uniformly isodiametric. It is not certain whether these structures appear in all sporogenous branches at some time preceding the contraction of the delimited protoplasts, or are more or less accidental in their occurrence. They also are found associated with septa in the sterile axial filaments, and here similarly occupy local hyphal distensions. After the individual spores have become separated, the connecting segments of evacuated hyphal wall contract slightly to form somewhat narrowed isthmuses, which persist until the mature spore chains are disrupted. In germinating, the spore usually produces 1 or 2 germ tubes.

The preparation from which figs. 92–101 were drawn was derived from one of 5 organisms communicated by Mr. M. SHAPAVALOV, who writes that "all were tested in inoculation experiments in 1912–1913, and proved to be pathogenic." Three of the other organisms were found to be identical morphologically with the one figured in plate VIII, while the fifth did not produce an aërial mycelium sufficiently profuse to permit of a satisfactory microscopic examination, although the general appearance of the culture indicated that it also is identical with *Actinomyces* XVI.

ACTINOMYCES XVIII

CULTURAL CHARACTERS.—On glucose agar, growth meager; nutritive mycelium colorless; aërial mycelium slow to develop, first white, later showing slight discoloration; diffusible pigment absent. On potato agar, development very rapid; nutritive mycelium dark; aërial mycelium profuse, felty, bluish gray; guttation moderate; tyrosinase reaction vigorous.

MORPHOLOGY.—This species is characterized by an unusual degree of variability in its fructifications. In figs. 102 and 107 is represented a relationship between axial filament and sporogenous branches common to many members of the genus. Fig. 108 shows a slight departure from this type in the thickening of the subterminal portion of the axial filament bearing the spiral branches. Further departures are expressed in the tufted grouping of the spiral hyphae in fig. 104, and in the distended and extremely vacuolated condition of the axial filament in fig. 106. A strikingly

aberrant type is seen in fig. 103, the fertile branches being short, inserted at close, irregular intervals, and showing no spiral tendency; while the axial filament is thick and abounding in spherical structures containing either deposits of metachromatic material or a partial equatorial septum.

In the dextrorse spiral hyphae of 1–8 open turns, 2.0–3.0 μ in diameter, the ellipsoidal spores, 0.8–0.9 $\times$ 1.0–1.6 μ, are produced by the insertion of conspicuous septa, sometimes in association with spherical structures. The presence of the latter (fig. 106), however, is not here indicated by local distensions. Subsequently the cross-walls undergo constriction and conversion to narrow connecting isthmuses. In the aberrant fertile hyphae (those without any spiral tendency), sporogenesis appears to take place in a more miscellaneous manner. Definite septa can rarely be distinguished, the spores seeming to result from protoplasmic contractions.

Isolated once from soil collected in Cambridge, Massachusetts.

Summary

1. The vegetative thallus of *Actinomyces* consists of a mycelium composed of profusely branching hyphae, the terminal growing portions of which are densely filled with protoplasm. Toward the center of the thallus the vacuoles increase in size and may be associated with the presence of metachromatic granules, the latter having nothing in common with bacterial endospores or "micrococci," for which they were mistaken by early observers.

2. The vegetative mycelium attains an extent incomparably greater than the branching figures recorded for bacteria of the acid-fast group, and the hyphae lack the uniformity in diameter generally characteristic of the Schizomycetes.

3. The aërial mycelium produced on suitable substrata by most species occurs usually in the form of a mat of discrete fructifications; but in other species these fructifications are frequently combined to form numerous and peculiar erect Isarioid sporodochia.

4. In any case each individual fructification represents a well characterized sporogenous apparatus, consisting of a sterile axial filament bearing branches in an open racemose or dense capitate arrangement. The primary branches may function directly as

sporogenous hyphae, or may proliferate branches of the second and of higher orders, sporogenesis in the latter case being confined to the terminal elements, the hyphal portions below the points of attachment of branches remaining sterile.

5. Two tendencies in the development of fructifications are recognizable: one leading to an erect drendroidal type, in which successively proliferated fertile elements undergo processes of sporogenesis in continuous sequence; and the other leading to a prostrate racemose type, in which sporogenesis is delayed in the older branches until the younger branches have also attained their final extension. The majority of species show these tendencies combined in different ways.

6. The sporogenous hyphae of most species are coiled in peculiar spirals, sometimes resembling the spores of the hyphomycetous genus *Helicoön*. These spirals exhibit pronounced specific characteristics in the number, diameter, and obliquity of their turns, and especially in the direction of rotation (whether dextrorse or sinistrorse).

7. Sporogenesis, where it can be followed, begins at the tips of the fertile branches and proceeds basipetally. In the larger number of species the process involves the insertion of septa which, in certain cases, are relatively very massive, and in others so thin as to be barely discernible. The disposition of these septa, while the delimited spores undergo maturation processes, varies with the species: (1) they may remain more or less unaltered; (2) they may suffer a median split, the two resulting halves being then separated as the result of the longitudinal contraction of the young spores, leaving alternate portions of hyphal walls completely evacuated; or (3) they may first become considerably constricted and subsequently converted into non-stainable isthmuses connecting the mature spores. The apparent absence of septa in the sporogenous hyphae of other forms is perhaps attributable to optical difficulties.

8. Granules are readily differentiated in the spores of many species which possess the staining properties and uniformity of size characteristic of nuclei; they generally occur singly, but in the larger spores of a few forms two are often found occupying diagonally opposite positions.

9. As in the vegetative thallus, metachromatic granules occur in the aërial mycelium, being very rarely found in the spores or sporogenous hyphae, but becoming very abundant in degenerate sterile hyphae.

10. The older axial filaments of some species show marked distensions which, in extreme cases, result in figures simulating *Leptomitus*. These arise as local distensions at the points of attachment of the more extensive lateral sporogenous processes. Cuneate modifications of the sterile axial filaments below the origins of branches also occur.

11. Curious spherical structures appear regularly in some forms, both in the sterile axial hyphae, where they may contain either a median septum or a number of peripheral metachromatic granules, and in the sporogenous hyphae, where they are associated with the regularly spaced septa.

12. The spores germinate readily in suitable solutions, producing 1–4 germ tubes, the approximate number being more or less characteristic of the species.

13. Owing to the absence of any well defined bacterial characteristics, the writer is of the opinion that the view that *Actinomyces* represents a transition between the Hyphomycetes and the Schizomycetes, as well as the phylogenetic corollary based upon it, may safely be abandoned. If mere size is to be regarded as important, it would appear to be equally profitable to look for bacterial affinities in some ascomycetous and sphaeropsideaceous forms, the hyphae of which are similarly very minute. It is doubtful whether far-reaching taxonomic generalizations can be based on the "acid-fast" staining reaction, especially as this reaction has not played a very important rôle in mycological research. There seems to be no adequate reason why the genus should not be classed in an unqualified manner with the Hyphomycetes, as a mucedineous group with tendencies toward an erect Isarioid habit.

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EXPLANATION OF PLATES II–IX

All except figs. 7, 8, 70, and 101, which are semidiagrammatical representations with a magnification of approximately 8000, were drawn with the aid of a camera lucida with a magnification of 2750.

PLATE II

Actinomyces I

FIG. 1.—Moderately well developed fructification.

FIG. 2.—Somewhat smaller fructification showing successive order of development: *a*, chain of spores, largely disrupted, developed from termination of axial hypha; *b*, *c*, *d*, secondary branches that have given rise respectively to series of elements *b1–b4*, *c1–c5*, and *d1–d3*.

FIG. 3.—Spore germinating with 4 germ tubes.

Actinomyces II

FIGS. 4–6.—Portions of aërial mycelium, showing conspicuous septa in fertile branches, and relation of latter to axial filaments: *a*, *b*, *c*, branches proliferated successively from same filament; *b1–b3*, *c1–c3*, elements proliferated successively from branches *b* and *c* respectively.

FIG. 7.—Portion of branch *c* (fig. 5) showing attachment of successively formed spiral elements.

FIG. 8*a–f*.—Successive stages in development of fertile hypha.

Actinomyces III

FIGS. 9–14.—Portions of aërial mycelium.

FIG. 15.—Portion of degenerate hypha containing abundance of metachromatic material.

PLATE III

Actinomyces IV

FIG. 16.—Short chain of spores showing nuclei, and 2 deep staining remnants of constricted septa in hyaline isthmuses between spores.

FIGS. 17–20.—Portions of aërial mycelium.

FIG. 28.—Spore germinating with 2 germ tubes.

Actinomyces V

FIG. 21.—Aërial hypha with spiral termination and 2 fertile branches, more mature elements showing failure of spore to develop in proximal portion.

FIG. 22.—Aërial hypha with 2 spiral elements.

FIG. 23.—Young spiral branch of 15 turns attached to axial hypha containing metachromatic granules.

FIGS. 24–26.—Spiral branches soon after insertion of septa, showing cross-walls absent from portion above basal septum.

FIG. 27.—Young spiral branch.

FIGS. 29, 30.—Spiral branches with spores mature, and non-septate portion completely evacuated.

FIGS. 31, 32.—Degenerate filaments containing much metachromatic material.

Actinomyces VI

FIG. 33.—Portion of aërial mycelium showing 2 spiral elements with nuclei in mature spores of one; septum in axial filament associated with basal septum in branch.

FIG. 34.—Similar to fig. 33, but without visible nuclei.

FIGS. 35–37.—Other portions of aërial mycelium.

Actinomyces VII

FIGS. 38–40.—Portions of aërial mycelium with sporogenous branches in various stages of development.

PLATE IV

Actinomyces VIII

FIG. 41.—Sporogenous apparatus with mature spores cohering in zoogloea-like masses.

FIG. 42.—Prostrate hypha containing numerous metachromatic granules and bearing a branch with many crowded spiral ramifications.

FIG. 43.—More open type of sporogenous apparatus with lateral elements attached to axial hypha at intervals.

FIG. 44.—Young sporogenous apparatus with spiral branches more or less crowded.

FIG. 45.—Somewhat older system of spiral hyphae, some of which have become converted into zoogloea-like masses of spores.

FIG. 46.—Lateral element bearing 8 spiral branches.

FIGS. 47, 48.—Portions of degenerate mycelium showing *Leptomitus*-like enlargements occupied by vacuoles, and metachromatic granules in constrictions.

FIGS. 49, 50.—Spores germinating with 1 germ tube.

Actinomyces IX

FIG. 51.—Portion of aërial mycelium showing spiral termination converted into chain of uninucleated spores, and presence of remnants of septa in hyaline isthmuses.

FIG. 52.—Similar to fig. 51, but without indications of septa between spores.

FIG. 53.—Portion of aërial mycelium showing septa in axial filament above insertions of some sporogenous branches.

FIG. 54.—Sporogenous branches with portion below second turn of spiral conspicuously thickened.

FIG. 55.—Sporogenous branch of 11 turns.

FIG. 56.—Spore germinating with 1 germ tube.

FIG. 57.—Sporogenous branch of 15 close spiral turns.

Actinomyces X

FIGS. 58–60.—Portions of aërial mycelium.

FIGS. 61, 62.—Spores germinating with 1 and 2 germ tubes respectively.

PLATE V

Actinomyces XI

FIGS. 63, 64.—More or less erect fructifications terminating long prostrate filaments, showing origin of groups of sporogenous branches from local hyphal distensions occupied by conspicuous vacuole.

FIGS. 65, 66.—Intermediate portions of aërial mycelium.

FIG. 113.—Spore germinating with 1 germ tube.

Actinomyces XII

FIGS. 67, 68.—Erect fructifications terminating long prostrate aërial filaments, exhibiting a pronounced tendency toward successive type of development, and showing cuneate hyphal enlargements below insertions of branches.

FIG. 69.—Intermediate portion of aërial mycelium.

FIG. 70*a–e*.—Progressive stages in development of sporogenous hypha, occurring in this and numerous other species.

FIG. 71.—Spore germinating with 3 germ tubes.

PLATE VI

Actinomyces XIII

FIG. 72.—Two portions, *a* terminal, *b* subterminal, of one long, unbranched, continuous sporogenous hypha showing very slight spiral tendency.

FIG. 73.—Chain of spores with deep staining polar granules.

FIGS. 74, 75.—Portions of aërial mycelium showing branching.

Actinomyces XIV

FIGS. 76–81.—Portions of aërial mycelium showing arrangement of sporogenous branches on hyphae, and method of sporulation.

PLATE VII

Actinomyces XV

FIG. 82.—Portion of aërial mycelium: elements *a1–a3* and *b1–b3* successively proliferated from branches *a* and *b* respectively.

FIG. 83.—Sporogenous branch *a* with 2 secondary branches; younger (*a3*) associated with a septum above insertion (successive type); older (*a2*) not set off by septum.

FIG. 84.—Axial filament with 3 branches, bearing successively poliferated elements *a1–a3*, as well as branch *ax*, latter not associated with septum in primary branch above point of attachment.

FIG. 85.—Fructification developed entirely in successive sequence, with 2 chains of uninucleated spores.

FIGS. 86–88.—Spores germinating with 2 germ tubes.

Actinomyces XVI

FIG. 89.—Large fructification consisting of axial filament *a–a1*, with whorl of 5 primary branches *a2–a6*, each bearing 1 or more secondary branches.

FIG. 90.—Spiral termination of sporogenous branch.

FIG. 91.—Old filament containing many metachromatic granules.

PLATE VIII

Actinomyces XVII

FIG. 92.—Portion of aërial mycelium showing spherical structures associated with septa in local distensions of sporogenous branch.

FIGS. 93, 94.—Portions of aërial mycelium, some lateral elements bearing secondary branches (indicated by numerals above 1) developed successively; 94*c*, unusually long fertile branch.

FIG. 95.—Portion of aërial mycelium similar to one shown in fig. 92.

FIGS. 96–100.—Spores germinating with 1 or 2 germ tubes.

FIG. 101*a–e*.—Successive stages in development of sporogenous branch, *cx* and *cy* representing either alternative or probably successive stages.

PLATE IX

Actinomyces XVIII

FIG. 102.—Sporogenous branch of usual type soon after appearance of septa.

FIG. 103.—Portion of fructification bearing aberrant fertile branches without spiral terminations.

FIG. 104.—Aërial filament with several spiral branches borne terminally.

FIG. 105.—Chain of mature spores developed from branch of spiral type.

FIG. 106.—Degenerate axial filament containing large vacuoles and spherical structures and bearing a fertile spiral branch.

FIGS. 107, 108.—Portions of aërial mycelium showing fertile hyphae of spiral type.

FIGS. 109–112.—Spores germinating with 1 or 2 germ tubes.